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(54) **USE OF LIPASE IN BAKING**

VERWENDUNG VON LIPASE BEIM BACKEN

UTILISATION D'UNE LIPASE DANS LA CUISSON AU FOUR

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Description

[0001] The present invention relates to a method for preparing a dough and/or of a baked product made from dough by use of enzymes. Furthermore, the invention relates to a dough and a pre-mix for a dough comprising these enzymes. Finally, the invention relates to the use of an enzyme preparation in a method for the preparation of dough and/or a baked product prepared from the dough.

BACKGROUND OF THE INVENTION

[0002] In the bread-making process it is known to add bread-improving additives and/or dough conditioners to the bread dough, the action of which, inter alia, results in improved texture, volume, flavour and freshness of the bread as well as improved machinability of the dough.

[0003] In recent years enzymes have been found to be useful as dough conditioners and/or bread-improving agents, in particular enzymes such as amylases and proteases which act on components present in large amounts in the dough.

[0004] Lipase (EC 3.1.1.3) is an enzyme belonging to the glycerol ester hydrolases, which catalyzes hydrolysis of ester bonds in triglycerides. The use of lipases in the preparation of bread has been suggested for smoothening and thereby improving the texture of bread, but it has been concluded that when lipase is used alone other properties of the bread such as bread volume, elasticity of the crumb and mouth-feel are deteriorated (JP-A 62-285749). In fact, the use of lipase in the baking industry has been stated as undesirable (Gams, 1976).

[0005] JP-A 62-285749 discloses a method of making bread, in which lipase is added to dough in admixture with vital gluten and lecithin. By the addition of vital gluten and lecithin the undesirable effects of lipase are stated to be avoided or diminished.

[0006] EP 468 731 discloses the use of a bread-improver comprising the enzyme glucose oxidase, optionally in combination with other enzymes such as hydrolases. Lipase is mentioned as one example of such hydrolase.

[0007] In both of the above references baking trials are described, in which lipase is added alone without addition of any of the other improving agents described in these references. These baking trials serve the purpose of illustrating the dissatisfactory results obtained by use of lipase alone as compared to the results obtained when lipase is used in admixture with the other improving agents described in these references. In EP 468 731 the lipase used for these trials have not been specified, whereas the lipase used in JP-A 62-285749 is stated to be Talipase, apparently a lipase produced by a species of the genus *Rhizopus*. In the baking trials disclosed in JP-A 62-285749, the lipase has been used in an amount which corresponds to at least 2240 Lipase Units (LU)/kg of flour.

[0008] In US-Patent No. 3,368,903, *Johnson* *et al.* methods are disclosed for retarding the tendency of bread to become stale comprising adding a lipase preparation to a bread dough mixture. The lipase used is obtained from a plant or is of fungal origin, namely from a strain of *Candida cylindracea*.

[0009] JP-A-4,158,731, Fukuhara *et al.*, discloses an improving agent for leavened frozen dough containing a lipase of unspecified origin, and a bread-making process using leavened frozen dough characterized by adding said lipase to the bread ingredients.

DISCLOSURE OF THE INVENTION

[0010] It was therefore surprising to find that the use of lipase in combination with an α-anylase and/or xylanase, as defined in the claims, under suitable conditions may result in substantial improvements of dough as well as of baked products prepared from the dough. In particular, it was surprising to find that some of the properties, which in some of the above-cited references were stated to be deteriorated by the use of lipase (such as bread volume) in fact could be improved. The present invention is based on this finding.

[0011] Accordingly, in a first aspect the present invention relates to a method for preparing a dough and/or a baked product made from dough by adding enzymes, as defined in the claims, to the dough and/or to any ingredient of the dough and/or to any mixture of the dough ingredients. The Lipase Units are further defined in the Materials and Methods section below.

[0012] It has been found that lipase also exerts an advantageous effect in dough without any added fat or dough containing only low amounts of added fat. Accordingly, the the dough may be fat-free.

[0013] As far as the present inventors are aware the use of lipase in the preparation of dough and/or baked products without or only low amounts of added fat is attractive for the preparation of low-calorie dough and/or baked products.

[0014] In the present context, the term "fat" is intended to indicate any fat or lipid useful in the preparation of dough and baked products. Fats conventionally used for this purpose includes butter, margarine, shortening, oil, and the like, and may be of vegetable or animal origin or of a mixed vegetable and animal origin. The term "fat-free" as used herein is intended to indicate that the dough is substantially free from added fat.

[0015] In one particular embodiment the dough comprises an amount of added fat constituting at the most 3.5% by

weight of the flour component(s) present in the dough.

[0016] The term "improved properties" as used about the effect obtained on dough and/or baked products made from dough according to the present invention is intented to be understood broadly, i.e. to include any property which may be improved by the action of lipase (in comparison with properties obtained when no lipase has been added).

[0017] In particular, it has been found that addition of lipase, as defined in the claims, results in an increased volume and improved softness of the baked product. Also, an improved anti-staling effect is obtained, i.e. the crumb of the baked product become softer when lipase is added. Furthermore, the colour of the crumb of the baked product becomes more white when lipase is added to the dough. In addition, dough prepared by a method of the invention has been found to obtain an improved consistency, i.e. an increased softness and elasticity, which results in a more easily machinable dough. The machinability of dough is a critical parameter, e.g., in the industrial production of dough and baked products.

[0018] In further aspects the present invention relates to a dough or a pre-mix for a dough, which comprises a lipase and an α-amylase and/or xylanase as defined in the claims.

[0019] In a final aspect, the invention relates to the use of an enzyme preparation in a method for the preparation of dough and/or baked products made from dough, as defined in the claims.

DETAILED DESCRIPTION OF THE INVENTION

[0020] The main triglyceride content in conventional bread dough containing no added fat is found in the flour component(s) of the dough and constitutes typically about 1-3% by weight of the dough. It is contemplated that the lipase enzyme used in accordance with the present invention is able to reach and act on these low amounts of triglycerides, even though free water molecules are virtually absent in the dough.

[0021] It has been reported by Weegels and Hamer (1992), Bekes et al. (1992) and Bushyk et al. (1990) that lipids present in dough interact with specific gluten complex proteins to form lipid-gluten aggregates during dough preparation.

[0022] Without being limited to any theory, it is presently believed that lipase used in accordance with the present invention modifies the interaction between lipid and gluten protein reported in the above cited references and thereby improves properties of dough and baked products. Although the nature of the interaction between lipid and gluten is unknown, it is contemplated that lipase reduces a possible lipid-qluten over-aggregation in the dough by exerting a limited attack on the lipid component of the aggregates without, however, making a total degradation of the aggregates. This modification of lipid-gluten aggregates is believed to result in an improved gluten complex, and thus an improved dough consistency, an enlarged bread volume and a better crumb structure as compared to the properties obtained when no lipase is added.

[0023] Furthermore, it is believed that lipase forms an "in situ" emulsifier comprising mono- and diglycerides in dough, which emulsifier is responsible for the improved anti-staling effect observed in accordance with the invention.

[0024] For the preparation of dough and/or baked products comprising only low amounts of added fat it is preferred that the amount of added fat constitutes at the most 3% by weight of the flour component(s) of the dough, preferably at the most 2.8% by weight of the flour component(s), such as at the most 2.5% by weight, more preferably at the most 2.0% by weight of the flour component(s) of the dough, still more preferably at the most' 1.5% by weight, even more preferably at the most 1.0% by weight and most preferably at the most 0.5% by weight of the flour components) of the dough.

[0025] The microbial lipase to be used in the method of the invention is a lipase derived from a strain of *Humicola* spp., especially from a strain of *H. lanuginosa*. An example of such a lipase is the *H. lanuginosa* lipase described in EP 305 216.

[0026] The lipase may be obtained from the microorganism in question by use of any suitable technique. For instance, a lipase preparation may be obtained by fermentation of a microorganism and subsequent isolation by a method known in the art, but more preferably by use of recombinant DNA techniques as known in the art. Such method normally comprises cultivation of a host cell transformed with a recombinant DNA vector capable of expressing and carrying a DNA sequence encoding the lipase in question, in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture.

[0027] The DNA sequence encoding the lipase to be used may be of any origin, e.g. a cDNA sequence, a genomic sequence, a synthetic sequence or any combination thereof. Examples of suitable methods of preparing microbial lipases are described in, e.g. EP 0 238 023 and EP 0 305 216.

[0028] Normally, the enzyme preparation to be used in the present invention is be added in an amount which, in the dough, results in a lipase activity in the range of 10-50,000 LU/kg of flour. A lipase activity below 10 LU/kg of flour is believed to provide no substantial effect, while a lipase activity above 100,000 LU/kg of flour is believed to result in an over-modification of the dough, e.g. a dough which is too sticky.

[0029] It is preferred that the lipase preparation is added in an amount which, in the dough, results in a lipase activity in the range of 10-3000 LU/kg of flour, 10-2500 LU/kg of flour, 10-2100 LU/kg of flour or 10-2000 LU/kg of flour. Very

advantageous effects on dough and bread are obtained when using a lipase preparation in an amount corresponding to a lipase activity in the range of 250-2100 LU/kg of flour, such as 500-2100 LU/kg of flour or 250-1500 LU/kg of flour.

[0030] The enzyme preparation to be used in the present invention may advantageously be used in combination with other dough conditioners or bread improvers.

[0031] The enzyme preparation to be used in a method of the invention may further comprise a cellulase, a glucose oxidase (useful for strengthening the dough), e.g. a fungal glucose oxidase such as Novozym 358® (a A. niger glucose oxidase), a protease (useful for gluten weakening in particular when using hard wheat flour), e.g. Neutrase@, a peroxidase (useful fo improving dough consistency), a peptidase and/or a maltogenase. Thus, any other components present in the enzyme preparation may be of a different or the same origin as the lipase. Alternatively, one or more additional enzyme activities may be added separately from the enzyme comprising the lipase. The other enzymes are preferably of microbial origin and may be obtained by conventional techniques used in the art as mentioned above.

[0032] A generally observed drawback when using pentosanase for baking is that the dough obtains an undesirable stickiness. It has surprisingly been found that lipase may reduce or avoid said stickiness. Accordingly, in one embodiment of a method of the invention, lipase is advantageously used in combination with a xylanase. The xylanase is preferably of microbial origin, e.g. derived from a bacterium or fungus, such as a strain of *Aspergillus*, in particular of *A. aculeatus, A. niger* (cf. WO 91/19782), A. *awamori* (WO 91/18977), or *A. tubigensis* (WO 92/01793), from a strain of *Trichoderma*, e.g. *T. reesei*, or from a strain of *Humicola*, e.g. *H. insolens* (WO 92/17573). Pentopan® and Novozym 384® (both from Novo Nordisk A/S) are commercially available xylanase preparations produced by *Trichoderma reesei*.

[0033] In an other embodiment of a method of the invention lipase is advantageously used in combination with an α-amylase.The α-amylase is preferably of microbial origin, e.g. derived from a bacterium or fungus, such as a strain of *Aspergillus*, in particular of *A. niger* or *A.* oryzae, or a strain of *Bacillus*. Commercially available α-amylases suited for the present purpose are Fungamyl® (an *A. oryzae* α-amylase), Novamyl® (a *B. stearothermophilus* α-amylase, cf. EP 120 693), and BAN® (a *B. amyloliquefaciens* α-amylase) all available from Novo Nordisk A/S.

[0034] The lipase is used in combination with an other enzyme, wherein the dosage of the lipase is 10-50,000 LU/kg of flour. The other enzyme activities may be dosed in accordance with established baking practice. In this respect, a preferred dosage of xylanase is 5-5000 FXU/kg of flour and a preferred dosage of amylase is 5-500 FAU/kg of flour.

[0035] The xylanase activity FXU (Farbe-Xylanase-Units) and the α-amylase activity FAU may be determined by the procedure given in the Materials and Methods section below.

[0036] Besides the above mentioned additional enzyme activities a microbially produced lipase preparation may contain varying minor amounts of other enzymatic activities inherently produced by the producer organism in question.

[0037] The enzyme preparation to be used in the method of the invention may be in any form suited for the use in question, e.g. in the form of a dry powder or granulate, in particular a non-dusting granulate, a liquid, in particular a stabilized liquid, or a protected enzyme. Granulates may be produced, e.g. as disclosed in US 4,106,991 and US 4,661,452, and may optionally be coated by methods known in the art. Liquid enzyme preparations may, for instance, be stabilized by adding nutritionally acceptable stabilizers such as a sugar, a sugar alcohol or another polyol, lactic acid or another organic acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238 216.

[0038] Normally, for inclusion in pre-mixes or flour it is advantageous that the enzyme preparation is in the form of a dry product, e.g. a non-dusting granulate, whereas for inclusion together with a liquid it is advantageously in a liquid form.

[0039] As mentioned above, the lipase. is believed to form an in situ emulsifier and in one embodiment it is thus contemplated to serve as a substituent for emulsifiers which are normally used for improving dough extensibility and to a certain extent for improving the consistency of bread (making it easier to slice), as well as for improving the storage stability of the bread.

[0040] However, the lipase preparation may also be used alongside conventional emulsifiers so as to achieve a better improving effect, which can not be achieved by using one or two emulsifiers alone. Examples of such emulsifiers are mono- or diglycerides, e.g. DATEM and SSL, diacetyl tartaric acid esters of mono- or diglycerides, sugar esters of fatty acids, polyglycerol esters of fatty acids, lactic acid esters of monoglycerides, acetic acid or citric acid esters of monoglycerides, polyoxyethylene stearates, phospholipids and (for the preparation of low fat-containing dough) lecithin.

[0041] The enzyme preparation may be added as such to the mixture from which the dough is made, or may, alternatively, be added as a constituent of a dough conditioner and/or a bread-improving composition. The dough conditioner and/or the bread-improving composition may be any conventionally used composition, e.g. comprising one or more of the following constituents:

[0042] A milk powder (providing crust colour), an emulsifier (such as mentioned above), granulated fat (for dough softening and consistency of bread), and oxidant (added to strengthen the gluten structure; e.g. ascorbic acid, potassium bromate, potassium iodate or ammonium persulfate), an amino acid (e.g. cysteine), a sugar, salt (e.g. sodium chloride, calcium acetate, sodium sulfate or calcium sulfate serving to make the dough firmer) and (for the preparation

of low fat-containing dough) gluten (to improve the gas retention power of weak flours).

[0043] Typically, the dough conditioner and/or the bread-improving composition is added in an amount corresponding to about 1-5%, such as 1-3% of the added flour.

[0044] The method of the present invention is contemplated to be useful in improving the machinability of any type of dough. Of course, the improved machinability is particularly important for dough types to be processed industrially, an example of which is dough types which are to be extruded (e.g. for the preparation of bisquits or crisp bread types). The use of lipase in such types of bread are believed to have no influence on the texture of the resulting product which means that no undesirable softening of products which are to be crisp are obtained. Furthermore, the use of lipase is considered to have no negative influence on the flavour of the resulting baked products, but is rather expected to improve the flavour.

[0045] As it is indicated above, the term "baked product" is intended to include any product prepared from dough. The baked product may be yeast-leavened or chemically leavened and may be of a soft or a crisp character. Examples of baked products, whether of a white, light or dark type, which may advantageously be produced by the present invention are bread, typically in the form of loaves or rolls, French baguette-type bread, pita bread, tacos, cakes, pancakes, bisquites, crisp bread and the like.

[0046] The dough and/or baked product prepared by the method of the invention are normally based on wheat meal or flour, optionally in combination with other types of meal or flour such as corn flour, rye meal, rye flour, oat flour or meal, soy flour, sorghum meal or flour, or potato meal or flour. However, it is contemplated that the method of the present invention will function equally well in the preparation of dough and baked products primarily based on other meals or flours, such as corn meal or flour, rye meal or flour, or any other types such as the types of meal or flour mentioned above.

[0047] As mentioned above the lipase preparation is added to any fixture of dough ingredients, to the dough, or to any of the ingredients to be included in the dough, in other words the lipase preparation may be added in any step of the dough preparation and may be added in one, two or more steps, where appropriate. However, the lipase should not be added together with any strong chemical or under conditions where the enzyme is inactivated.

[0048] The handling of the dough and/or baking is performed in any suitable manner for the dough and/or baked product in question, typically including the steps of kneading of the dough, subjecting the dough to one or more proofing treatments, and baking the product under suitable conditions, i.e. at a suitable temperature and for a sufficient periode of time. For instance, the dough may be prepared by using a normal straight dough process, a sour dough process, an overnight dough method, a low-temperature and long-time fermentation method, a frozen dough method, the Chorleywood Bread process, and the Sponge Dough process.

[0049] In a further aspect the present invention relates to a dough. The dough and the baked product prepared from the dough have improved qualities as defined above as compared with products which has not been prepared according to the invention. The baked product and the dough of the invention may be of any of the types discussed above, and it is preferred that the dough is fresh or frozen.

[0050] The enzyme preparation may be used in the form of a dough conditioner and/or a bread-improving composition which is substantially free from vital gluten or lecithin. These may be prepared on the basis of conventional dough conditioners and/or bread-improving compositions known in the art using procedures known in the art. Specific examples of suitable constituents for dough conditioners and/or bread-improving compositions are listed above.

[0051] The pre-mix of the invention may be substantially free from added fat. It may be prepared by techniques known in the art on the basis of pre-mix constituents known in the art such as flour, meal, dough-conditioners, bread improving additives and the like. Accordingly, in a further important aspect the present disclosure relates to the use of lipase in combination with an α-amylase and/or xylanase as a dough conditioner and/or a bread-improving agent for the preparation of dough and/or baked products substantially free from added fat or comprising at the most 3.5% of added fat by weight of the flour component(s) of the dough. .

[0052] When the dough contains added fat it is preferred that the amount of added fat constitutes at the most 3% by weight of the flour component(s) of the dough, such as at the most 2.8% or 2.5% by weight, more preferably at the most 2.0% by weight of the flour component(s) of the dough, such as at the most 1.5% by weight of the flour component (s), in particular at the most 1.0% by weight and most preferably at the most 0.5% by weight of the flour component (s) of the dough.

[0053] The present invention is further illustrated in the following examples which are not considered, in any manner, to limit the scope of the invention as defined herein.

MATERIALS AND METHODS

Enzymes

[0054] **Lipase A:** The *Humicola lanuginosa* lipase described in EP 305 216 and produced by recombinant DNA

techniques in *Aspergillus oryzae* as described in EP 305 216.

[0055] **Lipase B-I:** The *Rhizomucor miehei* lipase described by Boel et al. 1988 and produced by recombinant DNA techniques in *A. oryzae* as described by Huge-Jensen et al., 1989 and in EP 228 023.

[0056] The activity profiles of the above mentioned lipase enzymes appear from Table 1 below.

[0057] **Xylanase:** A xylanase produced by the *Humicola insolens* strain DSM 1800 available from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH and further described in EP 507 723.

[0058] **Fungamyl:** A commercial fungal α-amylase.preparation available from Novo Nordisk A/S.

Table 1

Lipase	LU/g	FAU/g
Lipase A	4,452,000	<0.6
Lipase B-I	12,200	9

LU/g (Lipase Units/g) and FAU/g (Fungal alpha-Amylase Units/g) were determined by the following assays:

LU - Lipase Units

[0059] Lipase activity was assayed using glycerine tributyrat as a substrate arid.gum-arabic as an emulsifier. 1 LU (Lipase Unit) is the amount of enzyme which liberates 1 µmol titratable butyric acid per minute at 30°C, pH 7.0. The lipaze activity was assayed by pH-stat using Radiometer titrator VIT90, Radiometer, Copenhägen. Further details of the assay are given in Novo Analytical Method AF 95/5, available on request.

FAU - Fungal alpha-Amylase Units

[0060] 1 FA-unit (FAU) is the amount of enzyme which at 37°C and pH 4.7 breaks down 5260 mg of solid starch per hour. Further details of the assay are given in Novo Analytical Method AF 9.1/3, available on request.

FXU - xylanase activity

[0061] The endo-xylanase activity is determined by an assay, in which the xylanase sample is incubated with a remazol-xylan substrate (4-O-methyl-D-glucurono-D-xylan dyed with Remazol Brilliant Blue R, Fluka), pH 6.0. The incubation is performed at 50°C for 30 min. The background of non-degraded dyed substrate is precipitated by ethanol. The remaining blue colour in the supernatant is determined spectrophotometrically at 585 nm and is proportional to the endoxylanase activity.

[0062] The endoxylanase activity of the sample is determined relatively to an enzyme standard.

[0063] The assay is further described in the publication AF 293.6/1-GB, available upon request from Novo Nordisk A/S, Denmark.

Preparation of bread

[0064] In Example 1 (comparative example), white bread were prepared from the following basic recipe:

Basic Recipe		
Wheat flour	1 kg	100%
Cold tap water	550 ml	55%
Fresh yeast	50 g	5%
Salt	20 g	2%
Margarine	60 g	6%

[0065] The wheat flour was of the type termed "Manitoba" supplied by "ValsemØlleme", Denmark, August 1991. The yeast was conventional yeast obtained from "De Danske Spritfabrikker" (Danisco), Denmark.

[0066] The cold water was added to a mixture of the dry ingredients. The resulting mixture was mixed for 3 minutes at 110 rpm and subsequently 8 minutes at 260 rpm using a spiral mixer, i.e. a Bear (BjØrn) Varimixer. The resulting dough was divided into portions of 350 g and subsequently allowed to rise for 40 minutes at room temperature and subsequently subjected to a second proofing in a proofing cabinet at 35°C and 70 RH for 50 minutes. Baking was

performed in tins at 225°C for 30 minutes.

[0067] In Examples 2-4 the following basic recipe and procedure was used:

Basic recipe	
Flour(Manitoba)	100 %
Salt	1.5 %
Yeast	5.0 %
Sugar	1.5 %
Water	54 %

Procedure

[0068]

1. Dough mixing (Spiral mixer)

   2 min. at 700 rpm
   7 min. at 1400 rpm

   the mixing time was determined and adjusted by a skilled baker so as to obtain an optimum dough consistence under the testing conditions used.

2. 1st proof: 30°C - 80% RH, 16 min.

3. Scaling and shaping;

4. Final proof: 32°C - 80% RH, 40 min.;

5. Baking: 225°C, 20 min. for rolls and 30 min. for loaf.

Evaluation of dough and baked products

[0069] Dough and baked products described in Example 1 were evaluated visually. The volume of the baked products was determined as further described below.

[0070] Properties of the dough and baked products described in Examples 2-4 were determined as follows:

[0071] **Roll specific volume:** The volume of 20 rolls are measured using the traditional rape seed method. The specific volume is calculated as volume ml per g bread. The specific volume of the control (without enzyme) is defined as 100. The relative specific volume index is calculated as:

$$\text{Specific vol. index} = \frac{\text{specific volume of rolls}}{\text{specific volume of control rolls}}$$

[0072] **Loaf specific volume:** The mean value of 4 loaves volume are' measured using the same methods as described above.

[0073] The dough stickiness and crumb structure are evaluated visually according to the following scale:

Dough stickiness	almost liquid	1
	too sticky	2
	sticky	3
	normal	4
	dry	5
Crumb structure	very poor	1
	poor	2
	non-uniform	3
	uniform/good	4

(continued)

	very good	5

[0074] The softness of bread crumb is measured by a SMS-Texture Analyzer. A plunger with a diameter of 45 mm is pressed on the middle of a 20 mm thick slice of bread. The force needed for the plunger to depress the crumb 5 mm with a speed of 2.0 mm/s is recorded and it is expressed as the crumb firmness. The lower the value, the softer is the crumb. Four slices of each bread are measured and the mean value is used.

EXAMPLES

EXAMPLE 1 (Comparative example)

[0075] White bread containing 6% of added fat were prepared on the basis of the standard procedure described above and the results shown in Table 2 were obtained:

Table 2

Lipase	Lipase B-I		
Dosage			
LU/kg of flour	10	100	1000
g/100 kg	0.1	1	10
FAU/100 kg	1	10	100
volume* (%)	101	105	111
Softness*			
Day 1	+	++	+++
Day 2	0	0	+
Effect on dough	**Some softening**		

* = relative to a reference without lipase added.

"0" means that the crumb is similar to that of the control, + means that the crumb is slightly softer, ++ means that the crumb is softer, and +++ means that the bread is considerable more soft than the control.
[0076] It is apparent that the use of a microbial lipase in the preparation of the dough and the baked product has a positive effect on dough softness as well as on the crumb structure and the volume of the baked product.

EXAMPLE 2 (Comparative example)

[0077] The enzyme used was Lipase A, i.e. a recombinant *H. lanuginosa* lipase. The enzyme was added either directly into the baking ingredients mix or it was dispersed in water before being added to the mix. All tests were carried out in duplicate and the results were similar. The following results were obtained:

Table 3

Lipase A	1	2	3	4	5	6	7	8
LU/kg flour	250	500	1000	1500	10000	20000	50000	- 0
Dough stickiness	All dough are good to work with.							
Roll specific vol index	119	124	122	122	113	111	103	100
Loaf specific vol index	111	112	118	112	111	108	104	100
Crumb structure	3,5	3	3,5	3,5	4	4	4	4
Softness/0	349	314	244	318	334	403	411	595
Softness/24 hr	705	541	559	627	606	656	735	1040
Softness/96 hr	988	1088	1049	1252	1091	1224	1261	1754

[0078] It is apparent from the above results that the addition of lipase increases the volume of rolls and loaves and improves the crumb structure and the crumb softness during storage. Furthermore, the lipase was found to have a significant effect on crumb whitness.

EXAMPLE 3

[0079] In order to evaluate whether lipase could advantageously be used in combination with other enzymes, baking tests were carried out with Lipase A, in combination with α-amylase. The α-amylase used was Fungamyl®.

[0080] The results obtained from the use of Lipase A in combination with Fungamyl® are given in Table 4 below.

[0081] From Table 4 it is apparent that bread prepared with Lipase A in combination with alpha-amylase have a larger volume and a better crumb structure than bread prepared with one of the enzymes alone. Furthermore, the addition of lipase reduce the dough stickiness which may normally be found, when a fungal α-amylase.is used alone for baking.

Table 4

	1	2	3	4	5	6	7	8	9	10
Lipase A/LU per kg flour	0	250	500	1000	1500	0	250	500	1000	1500
Fungamyl/FAU per kg flour	0	50	50	50	50	50	0	0	0	0
Dough stickiness	4	3	3,5	4	4	3	4	4	4	4
Roll sp. vol index	100	134	142	138	143	111	119	124	122	122
Loaf sp vol index	100	119	123	128	127	110	111	112	118	112
Crumb structure	3	4,5	4,5	5	5	4	3,5	3	3,5	3,5
Softness/0	398	264	263	300	275	265	356	446	308	293
Softness/24 hr	1076	464	557	493	482	532	864	879	659	628
Softness/72 hr	1727	1093	1002	806	1055	1214	1251	1301	1001	946

EXAMPLE 4

[0082] Pentosanases such as xylanase are know to have good baking effect. However, it is also commonly know that pentosanase can cause dough stickines. It was therefor tested whether lipase could be used to avoid or reduce the increased dough stickiness caused by the pentosanase xylanase, when used alone. More specifically, baking tests were performed (by use of the above stated general procedre) with Lipase A in combination with a *H. insolens* xylanase. The results obtained by use of Lipase A in combination with the xylanase are given in Table 5 below.

[0083] From Table 5 it is apparent that the stickiness caused by xylanase is reduced or disappeared, when this enzyme is used in combination with Lipase. Furthermore, the combination of lipase with xylanase ptovide larger volume, better crumb structure and less crumb staling than when lipase or xylanase is used alone.

Table 5

	1	2	3	4	5	6	7
Lipase A/LU per kg flour	0	250	500	1000	1500	0	0
Xylanase/FXU per kg flour	0	43	43	43	43	43	70
Dough stickiness	4	3,5	4	4	4	2,5	2,5
Roll sp vol index	100	136	143	134	140	120	127
Loaf sp vol index	100	116	122	119	119	108	118
Crumb structure	3	5	5	5	5	4	4,5
Softness/0	532	314	294	288	275	330	283
Softness/24 hr	1095	623	635	571	575	734	567
Softness/72 hr	1843	918	893	950	966	1370	1022

REFERENCES CITED IN THE SPECIFICATION

[0084] Bekes et al., Journal of Cereal Science 16, pp. 129-140, 1992

[0085] Boel et al., 1988, Lipids, Vol. 23, No. 7

[0086] Bushyk et al., "Carbohydrate and Lipid Complexes with Gliadin and Glutenin" in Gluten Proteins, Ed. W. Bushyk, American Association of Cereal Chemists, Minneapolis, 1990

[0087] Gams, 1976, Getreide Mehl und Brot, Technologische Zeitschrift für Getreide, Mehl, Brot, Backwaren, 30. Jahrgang, Heft 5, pp. 113-116
[0088] Huge-Jensen et al., 1989, Lipids Vol. 24, No. 9
[0089] Weegels, P.L. and Hamer, R.J., Cereal Foods World 1992, vol. 37, No. 5, pp. 379-385.

Claims

1. A method for preparing a dough and/or baked product prepared from the dough which comprises adding to the dough and/or any ingredient of the dough and/or to any mixture of the dough ingredients

   (a) a lipase derived from *Humicola* in an amount corresponding to a lipase activity of 10-50,000 LU/kg of flour, and

   (b) an α-amylase and/or xylanase.

2. The method of claim 1, wherein the lipase is derived from *Humicola lanuginosa*.

3. The method of claim 1 or 2, wherein the α-amylase.is derived from *Aspergillus*, more preferably from *A. oryzae*.

4. The method of any of claims 1-3, wherein the xylanase is derived from *Humicola*, more preferably from *H. insolens*.

5. The method of any of claims 1-4, wherein the lipase is added in an amount corresponding to a lipase activity of 10-3,000 LU/kg of flour, preferably 10-2,500 LU/kg of flour, more preferably 10-2,100 LU/kg of flour, even more preferably 10-2,000 LU/kg of flour, and most preferably 250-2,100 LU/kg of flour.

6. The method of any of claims 1-5, wherein the α-amylase is added in an amount of 5-500 FAU/kg of flour.

7. The method of any of claims 1-6, wherein the xylanase is added in an amount of 5-5,000 FXU/kg of flour.

8. The method of any of claims 1-7, wherein the dough comprises at the most 3.5% of added fat by weight of the flour component(s) of the dough.

9. The method of claim 8, wherein the dough is substantially free from added fat.

10. A dough or a pre-mix for a dough which comprises flour, a lipase derived from *Humicola* in an amount corresponding to a lipase activity of 10-50,000 LU/kg of flour and an α-amylase and/or xylanase.

11. Use of an enzyme preparation comprising a lipase derived from *Humicola* and an α-amylase and/or xylanase in a method for preparing a dough and/or a baked product prepared from the dough, wherein said lipase is added in an amount corresponding to a lipase activity of 10-50,000 LU/kg of flour.

12. The use of claim 11, wherein the lipase is derived from *Humicola lanuginosa*.

13. The use of claim 11 or 12, wherein the α-amylase is derived from *Aspergillus*, more preferably from *A. oryzae*.

14. The use of any of claims 11-13, wherein the xylanase is derived from *Humicola*, more preferably from *H. insolens*.

15. The use of any of claims 11-14, wherein the enzyme preparation is in the form of a non-dusting granulate or a stabilized liquid.

Patentansprüche

1. Verfahren zur Herstellung eines Teigs und/oder eines aus dem Teig hergestellten Backerzeugnisses, umfassend das Zusetzen zu dem Teig und/oder einem beliebigen Bestandteil des Teigs und/oder einem beliebigen Gemisch der Teigbestandteile

(a) einer Lipase, die aus *Humicola* stammt, in einer Menge, die einer Lipaseaktivität von 10-50.000 LU/kg Mehl entspricht, und
(b) einer α-Amylase und/oder einer Xylanase.

2. Verfahren nach Anspruch 1, wobei die Lipase aus *Humicola lanuginosa* stammt.

3. Verfahren nach Anspruch 1 oder 2, wobei die α-Amylase aus *Aspergillus,* stärker bevorzugt aus *A. oryzae,* stammt.

4. Verfahren nach einem der Ansprüche 1-3, wobei die Xylanase aus *Humicola,* stärker bevorzugt aus *H. insolens,* stammt.

5. Verfahren nach einem der Ansprüche 1-4, wobei die Lipase in einer Menge zugesetzt wird, die einer Lipaseaktivität von 10-3.000 LU/kg Mehl entspricht, vorzugsweise 10-2.500 LU/kg Mehl, stärker bevorzugt 10-2.100 LU/kg Mehl, und sogar noch stärker bevorzugt 10-2.000 LU/kg Mehl, und am stärksten bevorzugt 250-2.100 LU/kg Mehl.

6. Verfahren nach einem der Ansprüche 1-5, wobei die α-Amylase in einer Menge von 5-500 FAU/kg Mehl zugesetzt wird.

7. Verfahren nach einem der Ansprüche 1-6, wobei die Xylanase in einer Menge von 5-5.000 FXU/kg Mehl zugesetzt wird.

8. Verfahren nach einem der Ansprüche 1-7, wobei der Teig höchstens 3,5% zugesetztes Fett, bezogen auf das Gewicht der Mehlkomponente bzw. der Mehlkomponenten des Teigs, umfaßt.

9. Verfahren nach Anspruch 8, wobei der Teig im wesentlichen frei von zugesetztem Fett ist.

10. Teig oder Vormischung für einen Teig, umfassend Mehl, eine Lipase, die aus *Humicola* stammt, in einer Menge entsprechend einer Lipaseaktivität von 10-50.000 LU/kg Mehl, und eine α-Amylase und/oder Xylanase.

11. Verwendung einer Enzymzubereitung, die eine Lipase, die aus *Humicola* stammt, und eine α-Amylase und/oder Xylanase umfaßt, in einem Verfahren zur Herstellung eines Teiges und/oder eines aus dem Teig hergestellten Bakkerzeugnisses, wobei die Lipase in einer Menge zugesetzt wird, die einer Lipaseaktivität von 10-50.000 LU/kg Mehl entspricht.

12. Verwendung nach Anspruch 11, wobei die Lipase aus *Humicola lanuginosa* stammt.

13. Verwendung nach Anspruch 11 oder 12, wobei die α-Amylase aus *Aspergillus,* stärker bevorzugt aus *A. oryzae,* stammt.

14. Verwendung nach einem der Ansprüche 11-13, wobei die Xylanase aus *Humicola,* stärker bevorzugt aus *H. insolens,* stammt.

15. Verwendung nach einem der Ansprüche 11-14, wobei die Enzymzubereitung in der Form eines nicht-staubenen Granulates oder einer stabilisierten Flüssigkeit ist.

Revendications

1. Procédé pour préparer une pâte et/ou un produit cuit préparé à partir de la pâte, qui comporte l'ajout à la pâte et/ou à tout ingrédient de la pâte et/ou à tout mélange des ingrédients de la pâte :

(a) d'une lipase dérivée de *Humicola* selon une quantité correspondant à une activité lipase allant de 10 à 50000 LU/kg de farine, et
(b) d'une α-amylase et/ou d'une xylanase.

2. Procédé selon la revendication 1, dans lequel la lipase est dérivée de *Humicola lanuginosa.*

3. Procédé selon la revendication 1 ou 2, dans lequel l'α-amylase est dérivée de *Aspergillus,* de manière plus préférée

de *A. oryzae*.

4. Procédé selon l'une quelconque des revendications 1 à 3, dans lequel la xylanase est dérivée de *Humicola*, de manière plus préférée de *H. insolens*.

5. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel la lipase est ajoutée selon une quantité correspondant à une activité lipase allant de 10 à 3000 LU/kg de farine, de préférence de 10 à 2500 LU/kg de farine, de manière plus préférée de 10 à 2100 LU/kg de farine, de manière encore plus préférée de 10 à 2000 LU/kg de farine, et de manière la plus préférée de 250 à 2100 LU/kg de farine.

6. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel l'α-amylase est ajoutée selon une quantité allant de 5 à 500 FAU/kg de farine.

7. Procédé selon l'une quelconque des revendications 1 à 6, dans lequel la xylanase est ajoutée selon une quantité allant de 5 à 5000 FXU/kg de farine.

8. Procédé selon l'une quelconque des revendications 1 à 7, dans lequel la pâte comporte au plus 3,5 % de matières grasses ajoutées par poids du ou des composants de farine de la pâte.

9. Procédé selon la revendication 8, dans lequel la pâte est pratiquement exempte de matières grasses ajoutées.

10. Pâte ou prémélange pour une pâte qui comporte de la farine, une lipase dérivée de *Humicola* selon une quantité correspondant à une activité lipase allant de 10 à 50000 LU/kg de farine et une α-amylase et/ou une xylanase.

11. Utilisation d'une préparation enzymatique comportant une lipase dérivée de *Humicola* et une α-amylase.et/ou une xylanase dans un procédé pour préparer une pâte et/ou un produit cuit préparé à partir de la pâte, dans laquelle ladite lipase est ajoutée selon une quantité correspondant à une activité lipase allant de 10 à 50000 LU/kg de farine.

12. Utilisation selon la revendication 11, dans laquelle la lipase est dérivée de *Humicola lanuginosa*.

13. Utilisation selon la revendication 11 ou 12, dans laquelle l'a-amylase est dérivée de *Aspergillus*, de manière plus préférée de *A. oryzae*.

14. Utilisation selon l'une quelconque des revendications 11 à 13, dans laquelle la xylanase est dérivée de *Humicola*, de manière plus préférée de *H. insolens*.

15. Utilisation selon l'une quelconque des revendications 11 à 14, dans laquelle la préparation enzymatique a la forme d'un granulé non-poudreux ou d'un liquide stabilisé.